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Evaluation of Maternal Antibody Titers Against Viral Diseases and Hematological and Biochemical Characteristics in Different Lines of Indigenous Chicks

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ABSTRACT

The objective of this study was to examine the correlation between the maternal antibody titers (MAT) on the Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV), as well as various hematological and biochemical parameters, in five groups of one-day-old chicks. Following hatching, a total of 150 chicks were acquired, with 30 chicks obtained from each line. These chicks were not vaccinated and were subsequently divided into five groups, namely T1 representing black (BK), T2 representing nicked neck (NK), T3 representing bard (BD), T4 representing brown (BN), and T5 representing white (WT). The outcomes were analyzed statistically among all groups ($P < 0.05$). The results showed an increase in MAT against NDV was highest in WT group (13.649), while the decrease was the lowest in BK group (7.731). BK and BN recorded superior value in GOT concentration (21.0, 19.0, respectively). Group 3 (BD) demonstrated the most significant enhancements in MAT against IBDV, with a value of 10.833. This was followed by BK, WT, BN, and NK, which recorded values of 7.429, 6.852, 5.395, and 2.781, respectively. However, the various groups showed no significant variations in blood composition, except for heterophil, which exhibited significant differences across all groups. The values of MAT that were obtained from BK, WT, and BN were considered to be the most optimal, with respective measurements of 16.0, 19.0, and 20.0. The results found in this study indicated that there was a potential for genetic lines to influence the properties of maternal antibodies against NDV, IBDV, and other hematological parameters.

Keywords: Infectious bursal disease virus (IBDV), Maternal antibody titers, Newcastle disease virus, Serology.

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INTRODUCTION

Domesticated animals, particularly poultry, hold a prominent position in the generation of livestock revenue. This sector is widely recognized as a significant source of animal protein, encompassing meat and eggs, as well as byproducts that can be utilized in various transformative industries **(Nkukwana, 2018)**. According to **Alameri *et al.*, (2019)**, their study elucidated that the process of fowl domestication encompasses a significant portion, specifically 80%, of the chicken industry, with a particular focus on cost-effective dietary practices, predominantly observed in African and Asian regions. The indigenous chickens of Iraq exhibit a remarkable degree of adaptation to the local environmental conditions, particularly the arid and semiarid climate, in contrast to other commercially bred chicken varieties **(Tixier-Boichard and Weigend, 2020)**. They possess the most well-defined mechanisms for combating heat stress and disease. Additionally, these breeds exhibit a multitude of genetic variations and morphisms that are associated with underdeveloped regions, exclusion, and insufficient egg production due to their genetic constitution **(AL-Anbari, 2020)**.

In Iraq, indigenous chickens have emerged as a significant breed that warrants conservation due to their local adaptability and importance within the local poultry industry. However, poultry farming serves as a means of protein production, specifically through the production of eggs and meat. Additionally, it plays a significant role in generating income within rural communities **(Oljira, 2019)**. The genetic composition of a breeding population of Iraqi chickens was investigated by two Iraqi institutions, namely the indigenous chickens in Iraq **(Razuki and Al-Shaheen, 2011; Abdulwahid *et al.*, 2019; Abu-Rekaiba *et al.*, 2021)**. **AL-Anbari (2019)** achieved the development of six distinct genetic lines of indigenous chickens, specifically brown and black, white and striped, white

bare-necked, and brown bare-necked. In current studies, researchers have documented that indigenous chickens in arid and semiarid regions exhibit reduced productivity in terms of egg and meat production in comparison to foreign breeds and commercial strains **(Kanakachari *et al.*, 2022; Sibanda *et al.*, 2023)**.

Notwithstanding this, they demonstrate exceptional adaptability to the arid and semiarid climate, minimal requirements for care and management, brief feeding intervals, and robust resistance to prevalent parasites and diseases. According to **Hasselquist and Nilsson (2009)**, it was observed that maternal antibodies (MAB) are transferred from the hen to the offspring through the yolk of the egg. These antibodies are considered to be the initial protective barrier for newly hatched chicks, providing defense against common pathogens until their own immune systems fully develop. Up to at least 30% of IgY is being transferred to the digestive tract of baby chicks **(Ulmer-Franco, 2012; Rehan *et al.*, 2019)**. The kind of quality that antibodies from the hens have supports early-life sustenance in chicks. Whereas on the other hand, these antibodies prevent some categories of pathogenic agents **(Hamal *et al.*, 2006)**. A component that targets maternal immunity specifically is one of the differences noted with regards to the agents. For example, the efficacy of a live vaccine may be compromised for the first two weeks of life of an individual due to the presence of MAB. The elevated expression of MAB during this interval may inhibit the intended immunization response **(Sunwoo *et al.*, 2018; Deka *et al.*, 2020)**.

Maternal antibodies against NDV positively influence the reduction of live vaccine reaction intensity while simultaneously diminishing post-vaccination immunity. A high concentration of MAB against the IBDV neutralizes the vaccine virus, leading to total vaccination failure **(Gharaibeh and Mahmoud, 2013)**. Therefore, the hen's titer is highly applicable to be put in use by those clinicians in the field as an early predictor of the day-old chick's titer against specific pathogens in this perspective. This research work draws attention because of its collective design and the focus to quantify the antibody titers in newly hatched chicks. The main aim of these tests is to distinguish the differences between five local strains of Iraqi chickens in terms of maternal immunity against NDV and IBDV.Moreover, the study intends to find a relationship between some hematological and biochemical parameters with different local strains of Iraqi native chicken, hence collecting information relative to these traits that reside particularly to the Iraqi chicken.

MATERIALS AND METHODS

Experimental design

The study was approved by the Research Ethics Committee (Approval No. 274/2023), which recognized guidelines for animal experiments. The experiments that were conducted in this research adhered to the ethical standards set by the University of Anbar. The one-day-old chicks used in this investigation came from five different parental lines with varying genetic compositions. These chicks were obtained from the Research Station of Poultry, a division of the Ministry of Agriculture's Office of Agricultural Research. In the Poultry Research Station, a study was conducted on five different lines of chicks, namely T1 (black, BK), T2 (nicked neck, NK), T3 (bard, BD), T4 (brown, BN), and T5 (white, WT). Each line consisted of thirty-one-day-old chicks, and they were observed for their distinct phenotypic properties. All hens in the study adhered to the identical immunization schedule outlined in Table 1 and provided comparable management care for their offspring from one day old to 16 weeks old. Upon reaching 45 weeks of age, all parental subjects underwent an examination of their antibody titer in order to ascertain any discernible variations in the levels of antibodies present in the serum of the hens. Fertile eggs were collected twice daily over a period of ten days and subsequently stored at a temperature of 10°C until they were transferred to an incubator **(Table 1).**

Table 1: The implementation of a vaccination program for hens.

Age/ day	Vaccination name	Rote of administration	Manufacturer, country
1	Merck	Sub cutaneous	Merck Animal Health (MSD)
3	(Newcastle disease virus & infectious bursal disease virus) Diploid oil vaccine	Sub cutaneous	Creative Diagnostics, USA
7	Newcastle disease virus	Drinking water	Creative Diagnostics, USA
12-13	infectious bursal disease virus	Drinking water	IDEXX Laboratories, USA
17	Newcastle disease virus	Drinking water	Creative Diagnostics, USA
27	Newcastle disease virus	Drinking water	Creative Diagnostics, USA
42	Chickenpox	Injection into the wing fold	IDEXX Laboratories, USA
56	Newcastle disease virus	Drinking water	Creative Diagnostics, USA
112	(Newcastle disease virus & infectious bursal disease virus) Triploid oil vaccine & Egg drop syndrome	Sub cutaneous of neck	Creative Diagnostics, USA

Experimental chicks

A total of 150 chicks were obtained from the hatching process, with each line contributing thirty chicks. The individuals were exempted from receiving any immunizations until the end of the experiment. Upon departure from the hatchery, the chicks underwent a process of weight measurement using a digital scale in order to ascertain their initial weight, which ranged between 35 and 45 grams.

A blood sample was collected via acupuncture from the cardiac region utilizing an insulin syringe equipped with a 28.5-gauge needle and a volume of 0.5 ml. Blood samples were collected from 30 chicks from each lineage. The samples were divided into two groups: one group was collected in test tubes without anticoagulant for the purpose of analyzing serological parameters, while the other group was collected in test tubes with a coagulant to determine the blood composition.

Serological assays

The serum samples were collected following centrifugation at a speed of 3000 × g for 3 minutes. Subsequently, the samples were stored at a temperature of -20°C in order to assess the presence of MAB against NDV and IBDV. The ELISA kit utilized in this research was procured from the USA. Commercial ELISA kits evaluated serum antibody concentrations for IBDV and NDV. The instructions provided by the manufacturers were adhered to while utilizing the NDV antibody ELISA kit (Cat. No. DEIA1064, Creative Diagnostics, USA) and the IBDV antibody ELISA kit (Cat. No. 99-09260, IDEXX, USA). The manufacturer's protocol was followed, and the assay was performed using an automated reader microplate (ELx800, BIO-TEK Instruments Inc., Winooski, VT). The software provided by the manufacturer was utilized to quantify the antibody value for each individual sample. In each run, the comparative positive and negative control antisera were recorded for every sample of serum, following the guidelines provided in the basic recommendation kit, as described by **Snyder *et al.*, (1985)**.

Physiological assays

Biochemical assays include glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alanine aminotransferase (ALT) and glucose. Kinetic methods are applied to distinguish the activity of agents depending on the instruction from the Expert Panel of the IFCC (International Federation of Clinical Chemistry). Optical density was read at 450 nm by the Human company.

Blood was promptly liquefied by adding 2 mL of sterilized anticoagulant vials, in order to facilitate the measurement of white blood cells (WBC), red blood cells (RBC), lymphocyte percentage, and heterophil percentage.

Statistical analysis

In this study, all values were subjected to a one-way analysis in order to obtain variance across all procedures of the linear model using JMP IN 5.1 software. The statistical significance of the observed value was determined to be $p < 0.05$ across the entire dataset.

RESULTS

Table 2 provides a comprehensive overview of the titer of maternal immunity observed in five distinct strains of indigenous Iraqi chicks. The findings demonstrated statistically significant variations among all groups ($P < 0.05$). The WT group exhibited a greater enhancement in MAT against NDV (13.649), whereas BK demonstrated the lowest value compared to the other groups (7.731). Group 3 (BD) demonstrated superior enhancements in MAT against IBDV with a mean value of 10.833, followed by BK, WT, BN, and NK.

Table 2: Maternal antibody titers against NDV and IBD for five genetically indigenous Iraqi chicken breeds

Groups	NDV titer	IBD titer
BK	7.731 ± 0.0005^{e}	7.429 ± 0.02^{b}
NK	8.917 ± 0.001^{d}	2.781 ± 0.005^{e}
BD	9.351 ± 0.005^{c}	10.833 ± 0.001^{a}
BN	10.175 ± 0.005^{b}	5.395 ± 0.002^{d}
WT	13.649 ± 0.005^{a}	6.852 ± 0.001^{c}
LSD	0.02	0.05

BK representing black (T1), NK representing nicked neck (T2), BD representing bard (T3), BN representing brown (T4), and WT representing white (T5). $^{a\text{-}e}$ different letters refer to significant differences among groups ($p < 0.05$).

Table 3 presented the physiological characteristics of GOT, GPT, ALP, and glucose in various strains of local Iraqi chicks. As demonstrated, there were notable variations observed among the groups ($P < 0.05$). The concentrations of GOT were found to be higher in BK and BN, with values of 21.0 and 19.0, respectively. However, there were no significant differences ($P > 0.05$) between T2 and T3, both of which had the lowest values. NK exhibited a significantly higher value (296.0) in the GPT, greater than all other groups. The second group observed notable variations in the concentration of ALT, with a value of 246.0. However, there were no statistically significant differences ($P > 0.05$) between BD and BN. The initial group demonstrated an advantageous glucose value, with the lowest recorded measurement being 1082.0, while BD achieved a significantly higher value of 1632.

Table 3: Examined the biochemical blood parameters of five lines of local Iraqi chicks.

Groups	Biochemical parameters			
	GPT (IU/L)	GOT (IU/L)	Glucose (mg/dl)	ALP (U/L)
BK	218.0±0.57^{d}	21.0±0.57^{a}	1082.0±0.57^{e}	202.0±0.57^{d}
NK	296.0±0.57^{a}	13.0±0.57^{c}	1142.0±1.15^{d}	246.0±0.57^{a}
BD	249.0±3.5^{c}	11.0±0.57^{c}	1632.0±60.66^{a}	235.0±0.57^{b}
BN	208.0±0.57^{e}	19.0±0.57^{ab}	1243.3±1.21^{b}	233.3±0.88^{b}
WT	265.0±0.57^{b}	17.0±0.57^{b}	1195.0±0.57^{c}	227.6±0.33^{c}
LSD	10.6	3.6	4.48	3.87

BK representing black (T1), NK representing nicked neck (T2), BD representing bard (T3), BN representing brown (T4), and WT representing white (T5). $^{a-e}$ different letters refer to significant differences among groups (p <0.05).

Table 4 presents the absence of statistically significant differences within groups in terms of blood parameters such as RBC, WBC, and lymphocyte count. However, variations were observed among groups in terms of heterophil percentage.

Table 4: Examined the hematological blood parameters of five lines of local Iraqi chicks.

Groups	Blood parameters			
	RBC (10 ^{6}\µ I)	WBC (10^{3}\ µ I)	Lymphocyte%	Heterophils %
BK	2.5±0.05	12.0±0.57	45.0±0.57	16.0±0.57^{ab}
NK	3.0±0.57	12.0±0.57	50.0±2.8	15.0±0.57^{b}
BD	2.3±0.17	13.0±0.57	50.0±5.77	15.0±1.15^{b}
BN	3.5±0.28	12.0±0.57	45.0±2.88	20.0±1.15^{a}
WT	3.5±0.28	12.0±1.15	45.0±1.15	19.0±0.57^{ab}
LSD	NS	NS	NS	4.38

BK representing black (T1), NK representing nicked neck (T2), BD representing bard (T3), BN representing brown (T4), and WT representing white (T5). $^{a-e}$ different letters refer to significant differences among groups (p <0.05). NS, means non-significant.

DISCUSSION

Numerous chicken diseases result in elevated avian mortality and significant economic losses on farms. Newcastle Disease is regarded as one of the most harmful poultry diseases in developing nations. The incidence of ND was documented at 5.54% during the winter season and 4.16% in the summer **(Ullah *et al.*, 2019)**. Infectious bursal disease is one of the ailments introduced by indigenous and exotic breeds that leads to chicken mortality and decreased egg production, causing significant economic losses in poultry productivity **(Al-Obaidi *et al.*, 2024; Grace *et al.*, 2024)**. However, newly hatched chicks exhibit heightened susceptibility to various pathogenic agents due to their incomplete development of the immune system. The transfer of MAB from hens to chicks serves as a crucial mechanism for protecting against specific pathogenic factors, providing a vital form of early-age sheltering. According to **Sharma (1991)**, the assessment of serology in the diagnosis of a disease places significant emphasis on the measurement of inherited antibodies. The native chicken breeds of Iraq demonstrate remarkable resilience to the region's semi-tropical climate **(Al-Oubaidi *et al.*, 2020)**.

The robust defense mechanisms they possess against prevalent diseases, along with their possession of diverse genetic pools and numerous morphological variations that are influenced by the geographical diversity of the country, contribute to their adaptability **(AL-Anbari, 2020)**. **AL-Anbari (2019)** states that the

adoption of applied breeding programs is justified in order to improve performance outcomes and compensate for Iraq's substantial indigenous resources. The study conducted by **Fathi *et al.*, (2013)** revealed that strain and breed differences have a substantial influence on the transfer of antibodies from maternal sources to progeny, consequently affecting the development of the embryo. The spleen and bursa of Fabricius are anatomically linked in chickens via the genotype reservoir. The presence of the naked neck gene significantly reduces the weight value of chickens compared to their normal counterparts **(Adomako and Asamoah, 2025)**.

However, the presence of another gene (F) can increase the proportional weight of the bursa. The findings of the present study align with the conclusions drawn by **Taha and Aljumaily (2021)**, which omitted certain data points that pertained to the influence of male parental sex on the transmission of antibodies from parents to offspring in relation to NDV. Additionally, the genetic lineage and sex characteristics of the parent may still have a residual impact on the decline of MAB during the initial two weeks of the offspring's life. **AL-Anbari (2019)** addressed the observation that local chickens exhibit a higher level of immunity compared to other breeds in the country. This finding was utilized to enhance production capacity and exploit unique characteristics.

The serobiochemical parameters were utilized as biosignals to assess the extent of tissue degeneration. The study conducted by **Li *et al.*, (2019)** found that the health condition improved significantly, as indicated by the decreased levels of liver enzymes (GPT, GOT, and ALP) in the blood serum. This suggests that the organs were functioning optimally in the best-case scenario. Hepatic cells contain the GPT enzyme in both their cytoplasm and mitochondria **(Zhu *et al.*, 2020)**. The release of this substance into the bloodstream occurs specifically in response to substantial stress experienced by hepatic cells; thus, it functions as an essential marker for hepatic cell degeneration **(Zhang *et al.*, 2023)**. Pathogenic substances can prompt hepatocytes to secrete particular enzymes, such as GOT and GPT, which are then released into the bloodstream **(Elwan *et al.*, 2022)**. As a consequence, an elevated concentration of these enzymes is observed, serving as a reliable indicator of their activity. The condition of the liver is considered unsatisfactory on the basis of the bloodstream levels of GOT and GPT activity. The present investigation revealed discrepancies in biochemical parameters across five strains of indigenous Iraqi chicks, thereby establishing this study as a valuable asset for subsequent research.

The results of this study align with the findings of researchers who examined the biochemical characteristics of five local Iraqi female chicken breeds, revealing significant differences in HDL, cholesterol concentration, glucose levels, creatinine concentration, and triglycerides, which were attributed to the nutritional and health status of the birds **(Kamil *et al.*, 2020)**. This finding was similar to **Al-Dujaili *et al.*, (2021), who** documented significant ($p<0.05$) values of GPT and ALP in six strains of domestic roosters (local Iraqi breed); however, no significant values of GOT were observed among the rooster lines, with such differences attributed to the genetic distinctiveness of each species.

The findings of **Shanaw and Ghani (2022)** regarding the differences in WBC count between the local bare-necked white breed and brown were consistent with the obtained facts. Non-significant differences in WBC count were observed between two local Iraqi chicken breeds. However, these findings were not considered by **Isidahomen and Njidda (2012)**, who conducted research on the local naked neck chicken breed. In their study, they found that male chickens of this breed, which have curly and naturally feathered characteristics, exhibited significant improvement in WBC count. Several studies have scrutinized the productive characteristics of native Iraqi chickens, as recorded by **Al-Obaidi *et al.*, (2020); Al-Obaidi *et al.*, (2022) and Shanaw and Ghani (2022).**

Additionally, **Taha and Aljumaily (2021)** conducted a study on improving the genetic diversity of different types of native Iraqi chickens. This study investigated the impact of the structural characteristics, genetic composition, and sex of native avian species in Iraq, as compared to Brahman breeds, on different blood properties. All local bird groups, irrespective of gender or preference for diverse physiological feeds, have exhibited a discernible enhancement in quality, according to the findings of the study **(Bursali and Touray, 2024)**. The greater number of aphids in fig buds is indicative of the possibility that Brahma chickens are more adaptable to the climate of Iraq. The current study aligns with the findings of **Al-Dujaili *et al.*, (2021)**, which demonstrated significant differences in the mean values of Hb, PCV, and WBC counts among six strains of local Iraqi breeds. It concluded that the activities of bone marrow, liver metabolism, and renal endocrine function varied among different species of roosters raised under identical conditions and receiving the same nutritional level at the same age.

CONCLUSION

In conclusion, the data obtained from this investigation indicate the importance of considering genetic lineage in the maintenance of maternal antibody levels against NDV and IBDV. The results of this research could be utilized in commercial farms to assess

the antibody titers in one-day-old chicks, serving as an indicator of their hens' antibody levels. This study identified changes in biochemical characteristics among five lines of native Iraqi chickens; however, no differences were observed in the blood profile. Further investigations are required to identify the specific distinctions among varieties of indigenous Iraqi chicken.

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Conflict of interest

The authors declare that there is no conflict of interest

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